CONTRIBUTIONS TO THE LIFE-HISTORIES AND DEVELOPMENT OF *CUCULLANUS MINUTUS* RUDOLPHI, 1819 AND *C. HETEROCHROUS* RUDOLPHI, 1802 (NEMATODA : ASCARIDIDA)

BY

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CONTRIBUTIONS TO THE LIFE-HISTORIES AND DEVELOPMENT OF *CUCULLANUS MINUTUS* RUDOLPHI, 1819 AND *C. HETEROCHROUS* RUDOLPHI, 1802 (NEMATODA : ASCARIDIDA)

By D. I. GIBSON

CONTENTS

1. SYNOPSIS

The larval stages of the nematodes *Cucullanus minutus* and *C. heterochrous* from the flounder *Platichthys flesus* (L.) are described, those of *C. heterochrous* have not been previously recorded.

Information on the incidence of infestation of flounders with these larvae throughout the year is used to make a significant contribution to our knowledge of the life-histories of these two species. The work also indicates that *C. minutus* and *C. heterochrous* are normally geographically isolated from each other, and that this is associated with temperature and their life-histories.

2. INTRODUCTION

Cucullanus minutus and *C. heterochrous* were two of the commonest nematodes found in the flounder *Platichthys flesus* (L). from the estuary of the River Ythan, Aberdeenshire and from the sea off Aberdeen during the course of a survey of its helminth parasites. From previous work on the species of this genus, it appeared that the most obvious topics worthy of investigation were their life-histories and, especially in the case of *C. heterochrous*, the morphology of the larval stages. Janiszewska (1939) has described the third- and fourth-stage larvae of *C. minutus*, but did not determine any of this parasite's earlier life-history. With regard to *C. heterochrous*, no information on its life-history is available in the literature, and its larval stages do not appear to have been recorded previously. This is surprising considering how common and widespread these species are in flatfish.

A study of the literature revealed that there is little information on the life-histories of many members of the genus *Cucullanus*, and that no life-history has been satisfactorily explained throughout the complete cycle. The only explanation of a complete life-cycle is that of Vessichelli (1910) for *C. stelmioides* (Vessichelli, 1910). Vessichelli states that the eggs are laid and hatch in the gut-lumen of the lamprey *Petromyzon planeri* (Bloch). The larvae penetrate the gut-wall, encyst and remain there during the period of gut reduction before spawning. After spawning the lampreys perish, and lamprey larvae become infested by feeding upon the dead bodies of the adult lampreys. According to Shulman (1957) Vessichelli's work has not been confirmed and requires further investigation. Janiszewska (1939) showed that some of the larval stages of *C. minutus* were present in the gut-wall of flounders and that the final stage larvae migrated into the gut-lumen, but she could not ascertain how these larval stages came to be present in the gut-wall. Le-Van-Hoa & Pham-Ngoc-Khue (1967), when describing *C. chabaudi*, showed in the laboratory that the eggs of this species embryonate in two days, the first moult occurs inside the egg after four days and the second-stage larvae hatched from the eggs after five to six days. These authors then stated that the second-stage larvae continue to grow inside the swim-bladder of a fish (*Pangasius pangasius* Hamilton Buchanan), the second moult occurs in the liver, and the third and fourth larval stages are present in the gall-bladder and bile-duct. The fourth-stage larvae then migrate along the bile-duct to the intestine where the adult nematodes are found. These authors did not state how the second-stage infested the fish, and did not describe the larval stages. Finally, preliminary comments upon the life-histories of *C. minutus* and *C. heterochrous*, which are enlarged upon below, were made by MacKenzie & Gibson (1970).

3. METHODS

The mean incidence of infestation of the adult and larval stages of these two nematodes were obtained during a survey of the helminth parasites of the flounder. Seven hundred and forty flounders from the estuary of the River Ythan, Aberdeenshire (termed 'estuarine flounders') and one hundred and seventy flounders from the sea off Aberdeen (termed 'marine flounders') were examined. These results are represented in fig. 3. Most of the larvae were removed from the mucosa and sub-mucosa of the gut-wall by scraping, and the remainder deep in the sub-mucosa were found by examining sections of squashed gut-wall with transmitted light. The difference between mature and immature adult nematodes was taken to be, in the case of the females, the presence or absence of eggs, and, in the case of the males, the presence of clearly defined spicules when viewed under a low powered (× 10) microscope; but in most cases the mature and immature could easily be distinguished by size.

In the hatching experiments the adult worms were removed from the host, left over-night in 40% seawater, and the eggs produced were removed and kept in 80% seawater in a solid watch-glass.

4. RESULTS AND DISCUSSION

A. *Cucullanus minutus*

The only previous work on the life-history of this species was done by Janiszewska (1939) who described in detail the larval stages recovered from the gut-wall of flounders from the Baltic Sea. The free-living larvae have, however, never been described.

(i) Larval and adult development.

a. The egg:

Length :	61–68 μm
Breadth :	34–38 μm

The eggs were prolate spheroids and contained an unsegmented ovum when they left the female. The ova were seen to cleave into two- and four-cell stages, and then after two days the morula and blastula stages were visible. After four days a nematode larva was visible, coiled and moving within the egg-shell, but hatching did not take place until about seven days at 19°C. (133 degree days). Embryonation was very slow at low temperatures and was not observed below 7°C.

b. The first- and second-stage larvae:

Length :	304–374 μm
Breadth :	13 μm

The free-living larvae which hatched from the eggs (fig. 1) may have been the first- or second-stage larvae. No sign of a moulted cuticle was visible around the hatched larvae, though if such a cuticle had been tight fitting it may have been present. If this species resembles *C. chabaudi* (Le-Van-Hoa & Pham-Ngoc-Khue, 1967) then the first moult occurs within the egg, or if it resembles *C. heterochrous* (see below) the first larval cuticle is probably not lost until several days have passed in the free-living stage. Either way it is probable that the first- and second-stage larvae are very similar in appearance. Very little of the internal anatomy of these larvae was visible, though the anterior part of the oesophagus could be seen, showing that it was poorly developed compared with the adult. The remainder of the larva was obscured by the nuclei which, at this stage, are tightly packed together. These larvae were very active and capable of swimming. Unlike *C. chabaudi*, no second-stage larvae were ever recovered from the fish host.

c. The third-stage larva (fig. 1):

Length :	600–1,237 μm
Breadth :	40–80 μm
Oesophagus :	245–306 μm
Tail :	71–106 μm

This stage was described in detail by Janiszewska (1939) as the first larval stage from the intestinal wall. These larvae were situated in the mucosa and sub-mucosa of mainly the anterior intestine. The anatomy of this stage was as described by Janiszewska (1939), except that I believe that the thickening of the cuticle on the lips close to the mouth might in fact act as a boring tooth similar to that in the infestive stages of some anisakine nematodes.

d. The fourth-stage larva (figs 1 & 2):

Length :	720–1,210 μm
Breadth :	58–100 μm
Oesophagus :	278–346 μm
Tail :	80–106 μm

The fourth-stage larvae, or pre-adults, were described in detail by Janiszewska (1939) as the second larval stage from the intestinal wall. These larvae were found wandering through the gut-wall of the flounder feeding upon the tissues as they moved.

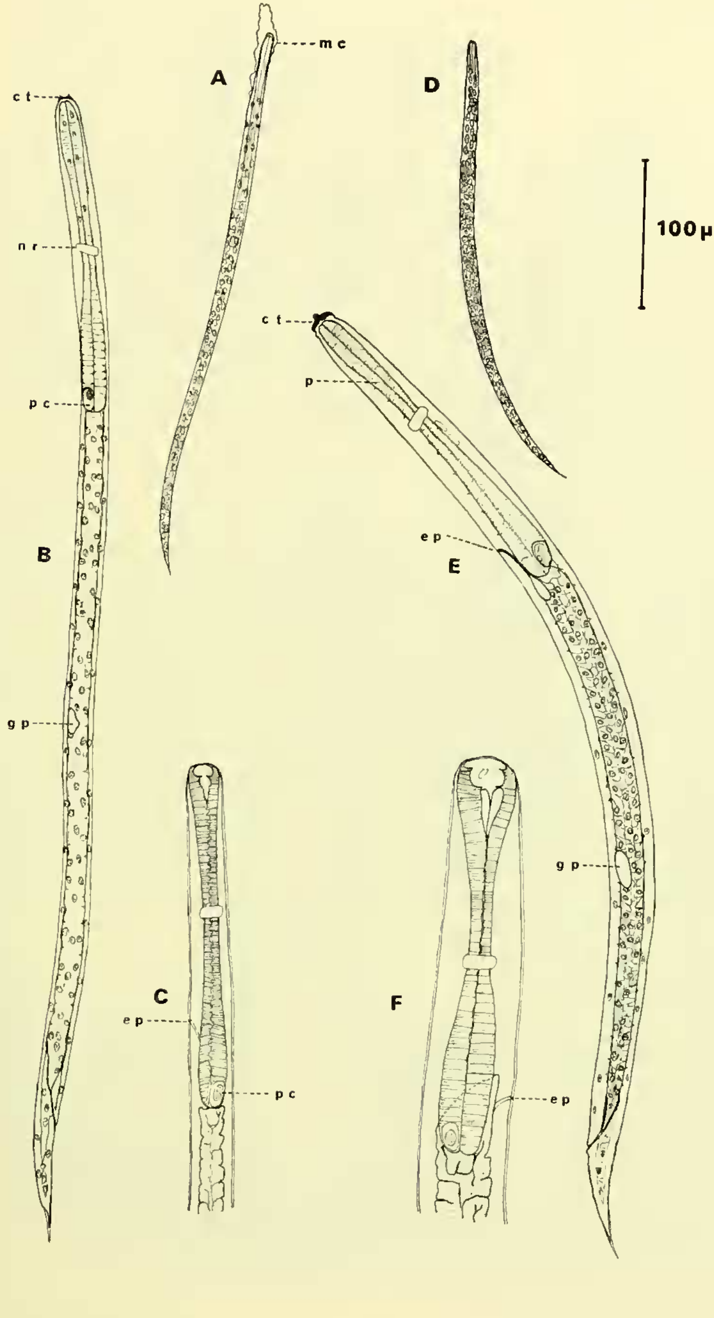
e. The immature adult (fig. 2):

Length :	600 (contracted) –2,100μm
Breadth :	113–332 μm
Oesophagus :	308–572 μm
Tail :	75–120 μm

These specimens were normally less than 2 mm. in length. It was not determined whether the final moult took place actually inside the body-wall or in the gut-lumen. The immature adults are similar in appearance to the mature adults, except that the sexual organs are not fully developed. The oral spines make their first appearance in the adult stage. These nematodes are capable of a great deal of contraction, and folds in the cuticle may form collars around the body. The behaviour of these immature adults is the same as that of the mature specimens.

FIG. 1. Cucullanid larvae. A. The second-stage larva of *Cucullanus heterochrous* within the first-stage larval cuticle. B. The third-stage larva of *C. heterochrous* from the flounder. C. The pre-adult of *C. heterochrous*. D. The first- or second-stage larva of *C. minutus*. E. The third-stage larva of *C. minutus* from the flounder. F. The pre-adult of *C. minutus*.

(ct, cuticular thickening; ep, excretory pore; gp, genital primordium; mc, moulted cuticle; nr, nerve ring; p, oesophagus; pc, large oesophageal cell)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f. The mature adult:

Length :	2,000–3,800 μm
Breadth :	210–515 μm
Oesophagus :	430–580 μm
Tail :	70–160 μm

The mature adults have been described in detail by Gendre (1926), Törnquist (1931) and Berland (1970), and figured by MacKenzie & Gibson (1970). The females were generally longer and in most cases broader than the males. The adults live by moving freely amongst and feeding upon gut-contents, or, when food is not available, by attachment to and feeding upon the gut-wall of the flounder. Attachment to the gut-wall is brought about by the sucking action of the muscular oesophagus, by the collarette and associated oral spines situated on the lips and possibly by the spiny nature of the pseudobuccal cavity. Aspects of the behaviour of this species were discussed by MacKenzie & Gibson (1970).

(ii) Life-history.

In flounders from the River Ythan third-stage larvae were present in small numbers throughout the year, but increased infestation occurred during the early spring (fig. 3), especially during March and April. Fourth-stage larvae were first found in March, but their numbers increased to a maximum during May and June and then fell to a very low level of infestation from August to November. Immature adults were first recovered in April, their numbers increased to a peak in June and July, and then their numbers fell in the autumn. Mature adults were first found in May, their numbers increased to a peak in July and August, and then fell off during the autumn with one or two individuals remaining until December.

In the marine flounders examined, the third-stage larvae were first recovered in January, the infestation had increased by March, but fell again by June (fig. 3). Fourth-stage larvae were first found in the March sample, the infestation was heavier in June, but had disappeared by September. Juvenile adults were recovered only from the June sample, and mature adults were found in the September and, to a greater extent, in the November samples.

(iii) Discussion.

The major period of egg-production of *C. minutus* in the River Ythan is during the summer months. This means that the eggs probably have time to embryonate and hatch during the late summer and autumn. This suggests that there is a gap of at least four to five months between the hatching of the eggs and the appearance of

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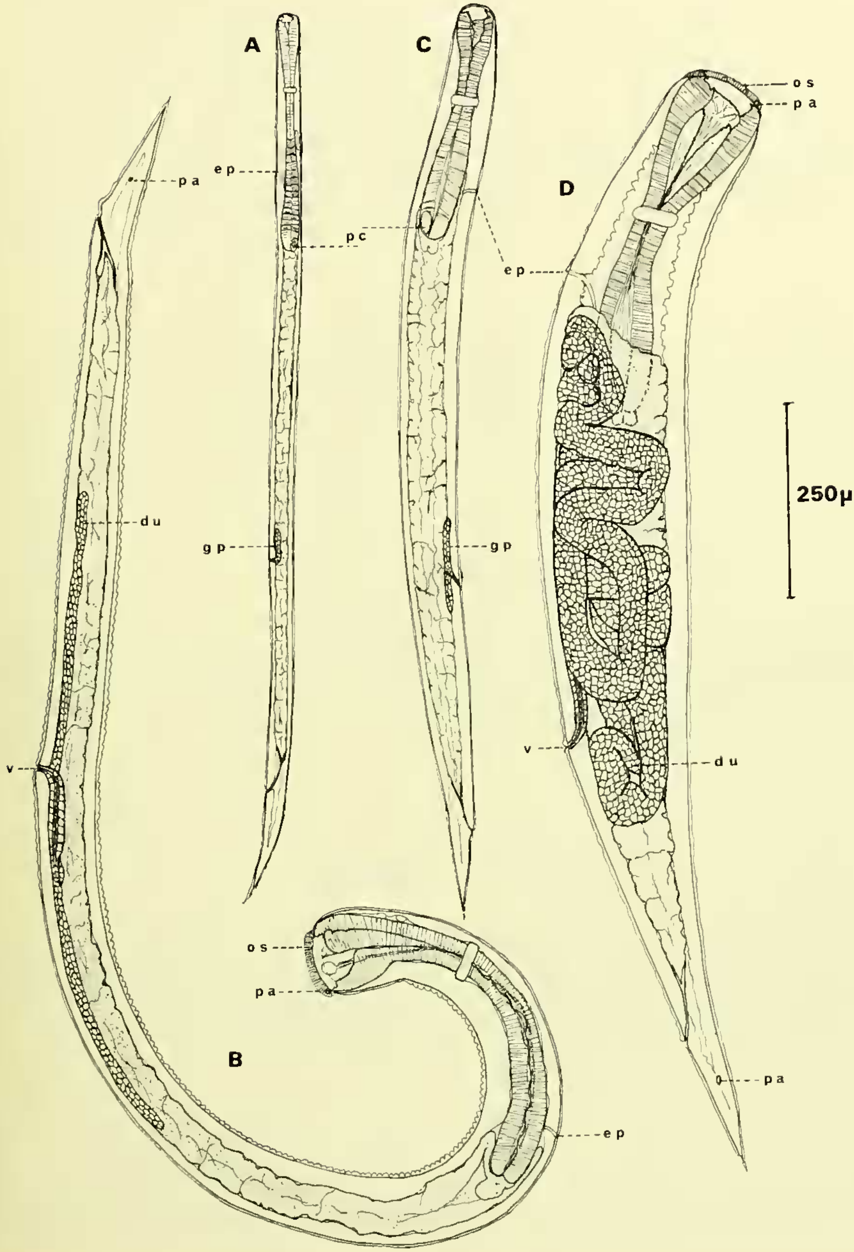
FIG. 2. Cucullanid pre-adults and immature adults (all drawings are of female worms). A. Pre-adult *Cucullanus heterochrous*. B. Immature adult *C. heterochrous*. C. Pre-adult *C. minutus*. D. Immature adult *C. minutus*.
(ep, excretory pore; du, developing uterus; gp, genital primordium; os, oral spines; pa, papilla; pc, large oesophageal cell; v, vulva)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the third-stage larvae in the flounders. As in the case of *C. heterochrous* discussed below, there are a number of possible explanations for this apparent gap : (1) an intermediate host is involved, (2) small second-stage larvae were too small to be seen during the flounder examinations, (3) there is an earlier site of infestation where the second-stage larvae develop and moult into the third-stage larvae, and (4) the second-stage larvae develop and grow in the free-living state. With regard to the first possibility, Wülker (1930) suggested that decapods and cumaceans were involved as intermediate hosts, but Janiszewska's (1939) attempts to infest such crustaceans all failed. Markowski (1966) suggested that *Nereis diversicolor* Müller might serve as an intermediate host for *C. minutus*, and I too suspected this animal because it occurs in large numbers in muddy estuaries. However, my attempts to infest this species have all failed. Similarly, I have failed to infest or find cucullanid larvae in *Neomysis integer* (Leach), *Corophium volutator* (Pallas), gammarids and *Crangon vulgaris* L., though some development in the free-living state may be necessary before the larvae become infestive. One of the main arguments for the presence of an intermediate host is the accumulation of the majority of the larvae in the anterior intestinal wall of the flounder. The possibility that the second-stage larvae are present in the gut-wall but have not been seen is unlikely because both Janiszewska (1939) and myself have failed to detect them. The possibility that there is an earlier site of infestation in the flounder cannot be overlooked, though none were found by Janiszewska (1939) or by myself, since Le-Van-Hoa & Pham-Ngoc-Khue (1967) did find such a site when studying *C. chabaudi*. However, as suggested below, the appearance of the third-stage larvae occurs during the period when most of the large flounders are at sea on their spawning migration. Therefore, if the flounders are being infested by the parasite at that time, this accounts for the fact that *C. minutus* tends to infest the small flounders more heavily than the larger ones (unpublished information) and also accounts for the absence of these larvae in O-group flounders (those less than one-year old). If an earlier infestation of the flounders does occur then alternative explanations must be found for these two results. Evidence for the final possibility, i.e. that the larvae live for some months in the free-living state, is not very forthcoming either, except that free-living larvae are produced when the eggs hatch and that these can be kept alive for more than a week in 80% seawater. As in the case of *C. heterochrous* discussed below, it is not yet possible to favour any of these alternatives too strongly, though the first and the last possibilities do seem more likely. It is possible, therefore, that the first stage-larvae moult into the second-stage larvae, which is probably the infestive stage, and these grow, with or without the use of an intermediate host, and infest the flounder mainly in the winter and early spring.

Janiszewska (1939) stated that the infestation of the flounders with the third-stage larvae commenced in August, which is earlier than the major period of infestation recorded in flounders from the River Ythan. This difference might be explained by the fact that on the coast of Poland, where Janiszewska (1939) was working, the sea will be warmer in summer than the waters of the River Ythan, and therefore development will be faster. The fact that temperature does affect the development of *C. minutus* is discussed below. In warmer waters, therefore, it is possible that infestation occurs in the autumn and mature specimens are found in the spring and summer, while in cooler waters the main period of infestation occurs in the early spring and mature adults are found in the late summer and autumn. In marine flounders from the Aberdeen area most of the mature adults were not found until the autumn, and this may account for the fact that third-stage larvae were not found in these fish in the autumn (fig. 3). The fact that the development of this parasite appears to take so long in marine flounders from the Aberdeen region may mean that in most cases the winter arrives and the parasite dies before egg production has really got underway. This could account for the small numbers of this parasite in these marine flounders.

B. *Cucullanus heterochrous*

The only previous comments on the life-history of this species were given by MacKenzie & Gibson (1970), and there is no previous record of the larval stages.

(i) Larval and adult development.

a. The egg:

Length : 75–102 μm
Breadth : 44–52 μm

The ova in the eggs cleaved in a similar manner to those of *C. minutus*, and larvae developed within the eggs after the same period of time. The eggs hatched after seven days at 19°C. and, like *C. minutus*, embryonation was not observed below 7°C.

b. The first-stage larva:

Length : 400–460 μm
Breadth : 12–14 μm

The first-stage larva developed within the egg, and after hatching was extremely active and capable of swimming. Any details of the anatomy of these larvae were difficult to see because of its small size and heavy concentration of nuclei. However, the anterior oesophagus could be distinguished in some specimens showing that it was very different from that of the adults because the musculature was much less developed.

c. The second-stage larva (fig. 1):

These larvae were so similar to the first stage-larvae that they could only be distinguished when parts of the moulted cuticle were still present. Such forms were found at 19°C. two days after hatching, though when the actual moult occurred is not known: this may even have been inside the egg. The oesophagus could be seen more clearly in the second-stage larvae, and was about 165 μm in length. The remainder of the alimentary canal could not be seen, and there was no anus visible.

d. The third-stage larva (fig. 1):

Length :	660–955 μm
Breadth :	25–27 μm
Oesophagus :	240–293 μm
Tail :	78–93 μm

These larvae were found encysted in the gut-wall of the flounder within small round cysts. I have regarded this stage as the third-larval stage because (1) it is parasitic, (2) it is much larger than the second-stage larva, (3) there are morphological differences between the second-stage larva and this larva, and (4) nothing resembling a second-stage larva was ever recovered from the flounder. The oesophagus of these larvae was better developed than in the previous stages, and the large oesophageal cells were visible, as they were in the corresponding stage of *C. minutus*. In the lip-region cuticular thickenings, which may act like boring teeth, were visible. In female specimens (fig. 1) a genital primordium could be seen, though, unlike the equivalent stage of *C. minutus*, I could not ascertain the position of the excretory gland or the excretory pore. The intestine appeared to be in the form of a tube composed of a large number of cells, and for the first time a definite anus could be seen.

The third-stage larva of the closely-related *C. cirratus* Müller, 1777, was recently described by Berland (1970); but this description resembles the fourth-stage larva of *C. heterochrous*. Berland's argument for calling this larva the third-stage larva was because the development of the gonads resembles the third-stage larva of *Contracaecum aduncum* (Rudolphi, 1802), but there is no vulva present in *C. minutus* or *C. heterochrous* until the fourth larval stage. Therefore, because of this and the structure of the head, I consider Berland's specimens to be fourth-stage larvae.

e. The fourth-stage larva (figs 1 & 2):

Length :	620–1,600 μm
Breadth :	27–40 μm
Oesophagus :	240–359 μm
Tail :	89–133 μm

In these larvae, which were observed moulting from the third-stage larvae within the cysts in the gut-wall, the cuticular thickenings on the lips were lost and the oesophagus became similar in shape to that found in the adults. The fourth-stage larvae, or pre-adults, left the cysts and were recovered wandering through the tissues of the gut-wall, feeding as they went. The lips possessed sharp edges which may have been associated with the movement through the tissues, because no oral spines or collarette were present at this stage. The large oesophageal cells were still visible, though they were not as clear as in the previous stage. In the female worms a developing uterus and vulva could be seen, though the vulva did not communicate with the exterior. The excretory pore was situated about half way between the nerve ring and the posterior edge of the oesophagus.

f. The immature adult (fig. 2):

Length :	1–6 mm.,	e.g. Length :	5,380 μm
		Breadth :	150 μm
		Oesophagus :	800 μm
		Tail :	173 μm

Oral spines and the collarette were present at this stage, and the pre-anal sucker and anal papillae were visible in males under a high powered microscope. In the females the uterus and ovaries were developing and the vulva could be seen to be connected to the exterior. These immature adults were found free in the lumen of the gut or attached to the gut-wall. It was not ascertained where in fact the final moult occurred or whether it was the pre-adults or the immature adults which migrated into the gut-lumen.

g. The mature adult:

Mature adults were described by Törnquist (1931) and Berland (1970). My specimens reached up to 12 mm., in the case of the females, and 10 mm. in length in the case of the males. These nematodes wandered through the intestinal contents of the flounder feeding as they went, though, like *C. minutus*, when food was not available they fed upon the gut-wall. Details of the migrations of these nematodes in the gut-environment have been given by MacKenzie & Gibson (1970).

In the past there has been some argument as to the nature of the structures which I have referred to as the oral spines. Gendre (1926), when discussing *C. minutus*, and Berland (1970) have commented that they may act as supporting 'ribs' for the cuticular collarette, and Berland (1970) also suggested that they may serve as an inter-locking device when the mouth is closed. My electron micrographs of *C. heterochrous* have shown that the spines, or denticulations, are, for at least the anterior-most part of their length, free of the collarette. They, therefore, probably aid the attachment of the parasite to the gut-wall, and may serve to split this wall and allow the muscular oesophagus to devour parts of the mucosa and sub-mucosa of the host.

(ii) Life-history:

The smallest larvae found in the flounders were the third-stage larvae. These were first recovered from the River Ythan in January, reaching a maximum in April, and then decreasing in numbers to zero in August, (Fig. 3). In marine flounders, however, these were found earlier, in November, when 8·8% of the fish examined contained small numbers of larvae. Due to the small size of these larvae small infestations may have been overlooked in the estuarine flounders, and this might account for this difference, but as mentioned below it is probable that there are no such larvae present in the Ythan estuary during these months. Fourth-stage larvae were first found in estuarine flounders during March. Their number increased to a maximum in May, and then decreased during the summer. In marine flounders their numbers increased during the late autumn and they were present in large numbers during the winter and spring. The dip in the level of infestation in marine flounders during the late winter may have been caused by the estuarine flounders entering the marine population at that time. Thus, as in the case of the third-stage larvae, the pre-adults are present in marine flounders long before they are in estuarine flounders.

Immature adults made their first appearance in the estuarine flounders during March, their numbers increased to a maximum intensity in June and a maximum incidence in July, and then their numbers decreased to zero by the following January. In marine flounders the numbers increased during the spring, as in Ythan flounders, but did not reach a peak until the autumn. This was possibly because the sea is cooler than the waters of the River Ythan during the summer months, and therefore development takes longer. These worms began to mature in estuarine flounders in September, mature adults increased in numbers to a maximum during the winter months and then decreased throughout the following spring. A similar effect was found in the marine flounders.

(iii) Discussion

The discrepancy in the periods of infestation with these larvae between the estuarine and the marine flounders could be the effect of temperature, since the waters of the River Ythan were warmer than the sea during the summer and cooler during the winter (H. D. Dooley, personal communication). However, there is a possibility that infestation with this parasite occurs much more frequently in the sea. This is suggested by the following facts: (1) infestation with this parasite is much heavier in marine flounders; (2) infestation with third-stage larvae proceeds earlier in marine flounders; and (3) this species tends to infest only the larger of the estuarine flounders (unpublished results) possibly because only the larger flounders participate in the spawning migration. It therefore appears that infestation of the marine flounders might occur before the arrival of the estuarine flounders in the sea, and the warmer temperature of the sea during the winter months probably allows faster development of these larvae in marine flounders. The sudden increase in the third-stage larval population in estuarine flounders in April is, therefore, probably associated with the return of some of the larger fish from the sea.

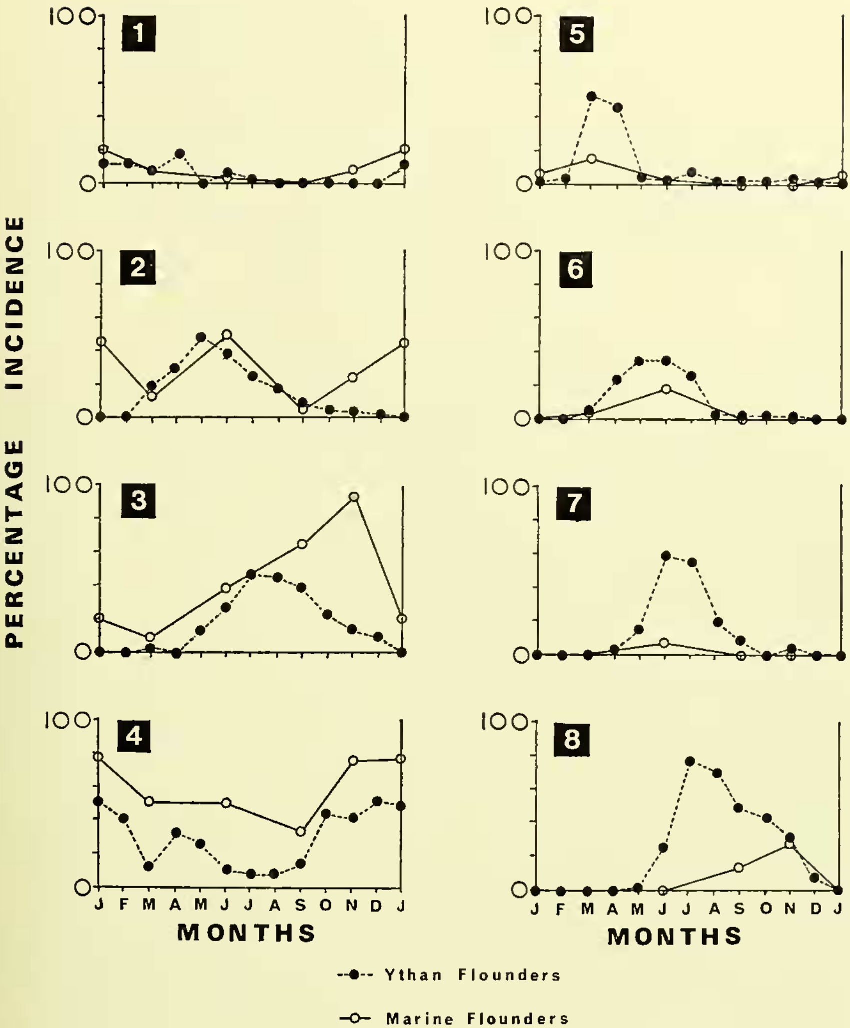

FIG. 3. The effect of season on the incidence of the larval and adult stages of *Cucullanus heterochrous* and *C. minutus* in flounders from the Ythan estuary and from the sea off Aberdeen. 1. The third-stage larvae of *C. heterochrous*. 2. Pre-adults of *C. heterochrous*. 3. Immature adults of *C. heterochrous*. 4. Mature adults of *C. heterochrous*. 5. Third-stage larvae of *C. minutus*. 6. Pre-adults of *C. minutus*. 7. Immature adults of *C. minutus*. 8. Mature adults of *C. minutus*.

The difference in size between the free-living second-stage larvae and the third-stage larvae recovered from the flounders suggests that: (1) smaller second- or third-stage larvae were missed during the examinations of the gut-wall; (2) there is an earlier site of infestation in the flounder; (3) there is an intermediate host; or (4) the second-stage larvae undergo a period of growth in the free-living state. Dealing with each point in turn, the first suggestion seems unlikely as no equivalent larvae were found by Janiszewska (1939) or myself in *C. minutus*. However, the second suggestion that there is an earlier site of infestation is a possibility, because Le-Van-Hoa & Pham-Ngoc-Khue (1967) found second-stage larvae of *C. chabaudi*, which migrated to the liver for the second moult, growing in the swim-bladder. It is therefore possible that such larvae might occupy a similar site in the body of the flounder. However, as stated below, it is most probable that most of the eggs hatch in the late spring and early summer, and therefore these larvae would be expected to infest the flounders at least by the autumn, long before the estuarine flounders enter the sea. Thus the high infestation in estuarine flounders returning to the estuaries in spring and the absence of larvae in O-group flounders cannot be explained by this suggestion, unless an earlier site of infestation is associated with the third and fourth suggestions. The third suggestion, that an intermediate host is involved, is doubtful because there is no evidence for the presence of such a host either in this species or in the other species of the genus discussed earlier. The life-cycle of the Camallanidae, nematodes with a similar mode of life, does in fact involve an intermediate host, but these are no longer thought to be closely related to the Cucullanidae (Inglis, 1967). The final suggestion, that the second-stage larvae grow in the free-living state, is suggested by the fact that free-living second-stage larvae were kept alive for more than a week in the laboratory. Both the third and the final suggestions would account for the apparent gap of several months between hatching and the appearance of the third-stage larvae in the flounders, the infestation acquired by the estuarine flounders when they entered the sea, and the absence of these larvae in O-group flounders. However, there is no further evidence for the latter suggestion, and, as stated by MacKenzie & Gibson (1970), the free-living larvae would penetrate neither the skin nor the gut-wall of the flounder. Though the larvae used for these latter experiments were recently hatched specimens, and it is possible that some development in the free-living stage is required before the larvae become infestive. It is clear that, at this stage, it is not yet possible to favour strongly any one of these suggestions until more information is available. Circumstances beyond my control hampered further work on this problem, but in my opinion the latter suggestion seems at present to be the most likely possibility, though the presence of an earlier site of infestation or possibility of an intermediate host cannot yet be overlooked.

In spite of the gap with regard to the actual method of infestation of the flounder, these results do give a good indication of what takes place during the life-history of this parasite. In my opinion, using the information given above, the life-history of *C. heterochrous* may occur as follows:

Most of the eggs are produced during the winter and early spring months, they hatch in the late spring and early summer when the water becomes warm enough

(7 °C.) and release free-living first-stage larvae. These require a number of days to moult into the second-stage larvae, which live on the sea-floor, grow and become infestive in the late autumn, winter and following spring. This coincides with the appearance of the third-stage larvae in the flounders. Most of these larvae moult into fourth-stage larvae (pre-adults) around April to May (though perhaps much earlier in marine flounders), and from then until June to July they grow inside the gut-wall feeding upon the tissues. During these latter two months many of the pre-adults moult into small immature adults, which, in the lumen of the gut, proceed to grow and slowly mature until the late autumn, winter and early spring when they begin to produce eggs. The mature adults continue to grow and produce eggs until their numbers decline in the late spring and summer. Nearly all the old adults are lost by the autumn.

5. FINAL DISCUSSION

In both *C. minutus* and *C. heterochrous* in flounders from the River Ythan the main period of apparent infestation coincides with the spawning migration. The fact that larvae of *C. minutus* tended to infest the smaller flounders and those of *C. heterochrous* the larger flounders (unpublished information) might be explained thus: *C. minutus* is a very common parasite of flounders from the Ythan estuary (Gibson, 1972), and as the smaller flounders do not take part in the spawning migration they are much more available to the parasite at this time, and, similarly, *C. heterochrous* is a very common parasite of flounders from the sea off Aberdeen (Gibson, 1972) and, as it is only the larger flounders which migrate into the sea, they will stand a better chance of becoming infested. It therefore appears that large flounders may migrate into the sea and become infested with *C. heterochrous* while the small flounders remain in the estuary and become more heavily infested with *C. minutus*.

In marine flounders *C. minutus* does not appear to develop to maturity until several months later than it does in estuarine flounders. This may be caused by the fact that the water-temperature of the Ythan estuary is warmer than that of the sea during the summer. The possibility that the development of this parasite is affected by temperature is also suggested by the fact that mature specimens were obtained from flounders at Plymouth in April, two months earlier than in flounders from the Ythan estuary. Further evidence can be deduced from the work of Markowski (1966), who found *C. minutus* in flounders from a brackish water reservoir containing water partially heated by the cooling water of a power-station, and in the sea a few yards away only a small number of flounders were infested with this parasite. When the zoogeography of this species is studied it is noticeable that *C. minutus* has not been found in northern waters except by Rudolphi (1819) and Janiszewska (1939) in the Baltic Sea. von Linstow (1904) reported *Dacnitis fusiformis* Molin, a synonym of *C. minutus*, from the Murman coast; but his specimens were from the rectum of the flounder and, as shown by MacKenzie & Gibson (1970), *C. minutus* is very rarely found in the rectum of the flounder, whereas *C. heterochrous* is most commonly found in that region of the gut. *C. minutus* has normally been found in a more southerly geographical area, e.g. Gendre (1926) in French estuaries, Stossich (1890, 1898) and Mola (1928) in the Mediterranean Sea, and Butzkaya (1952), Radulescu & Vasiliu-Suceveanu (1956), Bykhovskaya-Pavlovskaya et al. (1964), Komarova (1966), Markevitch (1967) and Naidenova (1970) in the Black Sea and its associated estuaries. On the other hand *C. heterochrous*, 'the winter species', has been recorded mainly in the North Sea, the Russian Arctic, the Siberian coast and the Gulf of the St. Lawrence, e.g. Nicoll (1907), Baylis (1928), Wülker (1930), Schuurmans Stekhoven (1935), Kreis (1952), Polyanski (1955), Zhukov (1960), Strelkov (1960), Berland (1961, 1970) and Ronald (1963). This species has been recorded further south only on a small number of occasions, e.g. Stossich (1892, 1898),* as *Dacnitis foveolatus* (Rud.), in the Mediterranean Sea and Gendre (1927) off the north-west coast of Africa. It therefore appears that *C. minutus* is a parasite of flatfish living in warmer water than *C. heterochrous*, and that Aberdeen is close to the northern limit of *C. minutus* in a marine environment and is in a position where the ranges of these two parasites overlap. Further north it is probable that, except in estuarine conditions which are warmer than the sea during the summer, *C. minutus* would not be capable of completing its full life-cycle in one year. The Baltic Sea may be an exception to this, as its isolation from the Atlantic Ocean and shallowness means that it is warmer in summer than equivalent latitudes of the North Sea, and, with its lower salinity, it is therefore suitable for the development of this parasite. The slow development and relatively long life of *C. heterochrous* contrasts with the fast development and short life of *C. minutus*, for if the above interpretations of the results are correct, the life-history of *C. heterochrous* takes two years for completion and that of *C. minutus* only one year. This may explain the fact that *C. heterochrous* is able to occupy a more northerly geographical range than *C. minutus*.

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* Stossich's specimens were not from flatfish and may therefore not have been *C. heterochrous*.

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